UNIVERSITY OF CALIFORNIA PUBLICATIONS
IN
AGRICULTURAL SCIENCES
Vol. 1, No. 3, pp. 39-49 December 14, 1912

THE EFFECTS OF CALCIUM AND MAGNESIUM CARBONATES ON SOME BIOLOGICAL TRANSFORMATIONS OF NITROGEN IN SOILS

BY
W. P. KELLEY

HISTORICAL INTRODUCTION

Loew[1] and his co-workers found, some years ago, that the growth of a number of plants may be markedly influenced by variations in the ratio of calcium to magnesium, both in solution and soil cultures. Osterhout[2] also showed that a more or less definite relation between other elements in culture solutions is necessary for maximum growth. These and other researches have drawn attention to certain long neglected phases of plant physiology and strengthen the view that in addition to the mere presence of the necessary elements, plants also demand a physiologically balanced relation between the elements in solution if maximum growth is to be produced. By means of artificial culture solutions principles of great importance are being worked out, but in generalizing from culture solutions to natural soils, many difficulties arise. The great complexity of the factors involved and the difficulties inherent in the question necessitate the greatest care in making broad generalizations regarding soils.

[1] Loew and May, Bur. Plant Ind. U. S. D. A., Bul. No. 1; Aso, Bul. Col. Agr. Tokyo, vol. 4, pp. 361–370; vol. 5, p. 495; vol. 6, p. 97; Loew and Aso, vol. 7, pp. 395–407.

[2] Bot. Gaz. 42, 127–134; 44, 259–272; 48, 98–104.

In this connection the "lime-magnesia ratio" has become a matter of general interest and is being extensively investigated at the present time. Some recent experiments by Lemmermann[3] and others seem to indicate that a wide variation in this ratio is of no consequence to plants. It is well known, however, that the effects produced by natural limes and limestones are not always equal. In certain instances dolomitic limes are known to produce less favorable results than non-magnesian limes. During recent years additional light on the action of lime in soils has been found in the fact that calcium carbonate enhances certain biological activities through supplying an active base by means of which the essential neutral condition is maintained. In this connection the question of the effects on bacterial activity brought about by different sources of lime and limestone naturally suggests itself.

In regard to physiologically balanced solutions for bacteria, Dr. C. B. Lipman[4] has shown that the ammonification of peptone by pure cultures of *B. subtilis* is favored on the one hand by a certain ratio of calcium to potassium, magnesium to sodium and potassium to sodium; while on the other hand, he failed to observe any antagonism between calcium and magnesium or calcium and sodium. In his investigations Lipman found that a certain concentration of magnesium chloride proved toxic to the development and activity of *B. subtilis* and at the same time the addition of certain amounts of calcium chloride failed to overcome this toxicity. Likewise, magnesium or sodium was ineffective in overcoming the toxicity of calcium. While it is probably true that calcium is not necessary for the normal development of bacteria, the importance of these observations, if found to apply in soils, is at once obvious.

From a study of the effects of various carbonates on the nitrification of ammonium sulphate in solutions, Owen[5] in 1908 concluded that magnesium carbonate is better suited to the stimulation and growth of nitrifying organisms than calcium, potas-

[3] Landw. Jahrb., 40 (1911), pp. 173–254; Also see Gile, Porto Rico Sta. Ann. Rept., 1911.

[4] Bot. Gaz., 48 (1908), pp. 105–125; 49 (1909), pp. 41–50.

[5] Ga. Sta. Bul. 81 (Technical Series No. 1), 1908.

sium or ammonium carbonates. It is but fair to mention in this connection, however, that great dilution of these carbonates was employed.

In 1910 Dr. J. G. Lipman[6] observed that the addition of one gram of calcium carbonate per one hundred grams of a New Jersey soil stimulated the ammonification of dried blood but depressed the formation of ammonia from cotton seed meal. In parallel experiments he observed that an equal amount of magnesium carbonate caused a depression in the ammonification of dried blood but stimulated the ammonification of cotton seed meal. In other words, the ammonification of dried blood and cotton seed meal in one and the same soil were affected by calcium and magnesium carbonates in opposite ways, both as regards the carbonates and the nitrogenous substances employed. These results are interesting and suggestive and point to the complexity of this single step in the preparation of available nitrogen from the organic substances occurring in soils.

In the same year Kellerman and Robinson[7] pointed out that the addition of magnesium carbonate to a highly magnesian soil in quantities above 0.25 per cent greatly depressed the formation of nitrates while the application of calcium carbonate in quantities up to 2 per cent markedly stimulated nitrification. The growth of crops on this soil had been found to be much more favorably influenced by the application of ground oyster shells than by magnesium limestone. The authors inferred from their experiments that the inferior effects on crops following the application of dolomitic limestone may be due, in part, to retarded nitrification.

AMMONIFICATION

In the course of some studies on soil bacteriology at the University of California, the writer undertook a study of certain biological transformations, as affected in two different sandy soils from California by varying amounts and combinations of calcium and magnesium carbonates. On account of the striking nature of the results obtained in the preliminary ammonification ex-

[6] N. J. Sta. Rept., 1910.

[7] Science, 32, p. 159.

periments a systematic study of this question was undertaken. The soil employed in the ammonification experiments presently to be described was of a light sandy character having been taken from near Oakley in the upper part of San Joaquin Valley and represents a large area now devoted to the growth of peaches and other fruits. With suitable moisture conditions this land produces excellent growth of the crops suited to it. The following analysis furnished by the courtesy of Dr. Lipman sets forth the composition of this soil.

TABLE I. COMPOSITION OF SOIL USED IN AMMONIFICATION EXPERIMENTS

	Per cent
Insoluble matter	80.45
Soluble silica	6.15
Potash (K_2O)	0.35
Soda (Na_2O)	0.15
Lime (Ca O)	1.41
Magnesia (Mg O)	0.33
Br. Ox. Manganese (Mn_3O_4)	0.09
Ferric Oxide (Fe_2O_3)	3.96
Alumina (Al_2O_3)	4.45
Phosphoric Acid (P_2O_5)	0.10
Sulphuric Acid ($S O_3$)	0.06
H_2O at 110° C.	0.80
Volatile matter	2.02
Total	100.32

In the ammonification experiments dried blood was used as a source of nitrogen. Five grams of this material and varying amounts of calcium and magnesium carbonates were thoroughly mixed with 100 gram portions of sifted soil, placed in tumblers and then optimum moisture conditions provided by the addition of sterile water. The tumblers were covered with Petri dishes and after an incubation period of seven days the ammonia was distilled into standard acid by the use of magnesium oxide and measured in the usual way. The results are recorded in the following table.

TABLE II. EFFECTS OF CALCIUM AND MAGNESIUM CARBONATES ON THE AMMONIFICATION OF DRIED BLOOD

Treatment	Ammonia nitrogen mgs.
None	81.4
1 Gram Calcium Carbonate	84.3
2 Gram Calcium Carbonate	85.0
4 Gram Calcium Carbonate	91.0
6 Gram Calcium Carbonate	91.0
8 Gram Calcium Carbonate	87.8
12 Gram Calcium Carbonate	87.8
1 Gram Magnesium Carbonate	53.2
2 Gram Magnesium Carbonate	53.9
4 Gram Magnesium Carbonate	50.0

These data, as all others submitted in this paper, represent averages of closely agreeing duplicates. In examining the above data we note a slight stimulation in ammonia formation from the use of the several amounts of calcium carbonate employed, the maximum stimulation being reached with from 4 to 6 grams per 100 grams of soil. With the use of magnesium carbonate a marked depression in ammonia accumulation occurred, there having been found to be a falling off of approximately one-third as compared with the amounts found without the use of carbonate. It is also noteworthy that one gram of magnesium carbonate proved to be about as toxic to ammonification as larger amounts.

A second series was prepared with the use of still smaller amounts of magnesium carbonate for the purpose of determining the concentration at which toxic effects begin and also to determine the minimum amount of this carbonate necessary to produce maximum toxicity. The results follow.

TABLE III. AMMONIFICATION OF DRIED BLOOD AS AFFECTED BY SMALL AMOUNTS OF MAGNESIUM CARBONATE

Treatment	Ammonia nitrogen mgs.
None	93.1
0.1 Gram Magnesium Carbonate	77.4
0.2 Gram Magnesium Carbonate	70.6
0.4 Gram Magnesium Carbonate	65.6
0.6 Gram Magnesium Carbonate	65.2
0.8 Gram Magnesium Carbonate	64.6
1.0 Gram Magnesium Carbonate	62.0

These data are instructive as showing the marked depression of ammonification in the soil employed, even with the small amount of .1 per cent of magnesium carbonate. The toxicity increased with greater amounts of the magnesium carbonate added reaching a practical maximum with from 0.8 to 1 gram per 100 grams of soil.

According to Loew the toxic effects of an excess of magnesia in soils can be overcome or antagonized by the application of lime. While this theory was proposed and held for the higher plants, it was thought to be of some interest to study the question with reference to the ammonification process. Accordingly the following series of experiments was arranged. In these trials one gram of magnesium carbonate per 100 grams of soil was used throughout, this quantity having been found to be the lowest that produced maximum toxicity.

TABLE IV. AMMONIFICATION OF DRIED BLOOD IN THE PRESENCE OF BOTH $CaCo_3$ AND $MgCo_3$

Treatment	Ammonia nitrogen mgs.
1 Gram Calcium Carbonate	84.3
1 Gram Magnesium Carbonate	53.9
1 Gram Magnesium Carbonate + 0.5 Grams Calcium Carbonate	51.1
1 Gram Magnesium Carbonate + 1. Grams Calcium Carbonate	53.9
1 Gram Magnesium Carbonate + 2. Grams Calcium Carbonate	53.2
1 Gram Magnesium Carbonate + 3. Grams Calcium Carbonate	50.6
1 Gram Magnesium Carbonate + 4. Grams Calcium Carbonate	51.1
1 Gram Magnesium Carbonate + 5. Grams Calcium Carbonate	50.7
1 Gram Magnesium Carbonate + 6. Grams Calcium Carbonate	50.3
1 Gram Magnesium Carbonate + 8. Grams Calcium Carbonate	50.7
1 Gram Magnesium Carbonate + 12. Grams Calcium Carbonate	50.4

From these data it is at once seen that no antagonism was produced. Even the very large amount of 12 grams of calcium carbonate in no way reduced the toxic effects produced by one gram of magnesium carbonate. The results, therefore, are in harmony with the observations made by Dr. C. B. Lipman[8] in his studies on the physiology of *B. subtilis.*

[8] *Loc. cit.*

NITRIFICATION

Having failed to observe any antagonism between calcium and magnesium in the complex process of ammonification in the soil under investigation, attention was directed to a study of nitrification under similar conditions. A sandy soil from Anaheim, California, that contained a vigorous nitrifying flora, was employed in these studies. The following table of analyses furnished by the kindness of Dr. Lipman shows the chemical composition of this soil.

TABLE V. COMPOSITION OF SOIL USED IN NITRIFICATION EXPERIMENTS

	Per cent
Insoluble matter	73.59
Soluble Silica	11.17
Potash (K_2O)	.64
Soda (Na_2O)	.15
Lime (Ca O)	1.39
Magnesia (Mg O)	.93
Br. Ox. Manganese (Mn_3O_4)	.04
Ferric Oxide (Fe_2O_3)	5.10
Alumina (Al_2O_3)	3.92
Phosphoric Acid (P_2O_5)	.12
Sulphuric Acid (SO_3)	.02
Volatile matter, H_2O at 110° C	2.88
Total	99.95

The nitrification experiments were carried out in tumblers, two grams of dried blood being mixed with each 100 gram portion of soil. The amounts of calcium and magnesium carbonates added are shown in the table. Optimum moisture conditions were maintained throughout the 21 day incubation period during which time a temperature of 27 to 28 degrees was maintained. The results are shown in the following table.

TABLE VI. EFFECTS OF CALCIUM AND MAGNESIUM CARBONATES ON THE NITRIFICATION OF DRIED BLOOD

Treatment	Nitrate nitrogen found mgs.
None	14.5
1.0 Gram Calcium Carbonate	23.5
2.0 Gram Calcium Carbonate	19.2
4.0 Gram Calcium Carbonate	21.2
8.0 Gram Calcium Carbonate	20.2
0.1 Gram Magnesium Carbonate	3.6
0.2 Gram Magnesium Carbonate	2.9
0.4 Gram Magnesium Carbonate	2.8
0.8 Gram Magnesium Carbonate	5.1
1.0 Gram Magnesium Carbonate	1.0
2.0 Gram Magnesium Carbonate	2.0
4.0 Gram Magnesium Carbonate	2.9
8.0 Gram Magnesium Carbonate	3.3
Original soil	5.0

It will be observed that while approximately a 50 per cent stimulation in nitrate formation was effected by the addition of calcium carbonate, nitrification was totally inhibited by the addition of one-tenth of one gram of magnesium carbonate. Before further discussing these results the data obtained from the effects of calcium and magnesium carbonates acting synchronously will be presented.

TABLE VII. THE LACK OF ANTAGONISM BETWEEN CALCIUM AND MAGNESIUM CARBONATES AS SHOWN IN THE NITRIFICATION OF DRIED BLOOD

Treatment	Nitrate nitrogen found mgs.
None	14.5
1.0 gram Calcium Carbonate	23.5
0.1 gram Magnesium Carbonate	3.6
0.1 gram Magnesium Carbonate and 1. gram Calcium Carbonate	4.1
0.1 gram Magnesium Carbonate and 2. gram Calcium Carbonate	3.4
0.1 gram Magnesium Carbonate and 3. gram Calcium Carbonate	2.6
0.2 gram Magnesium Carbonate and 1. gram Calcium Carbonate	1.9
0.2 gram Magnesium Carbonate and 2. gram Calcium Carbonate	1.4
0.2 gram Magnesium Carbonate and 3. gram Calcium Carbonate	2.0
0.4 gram Magnesium Carbonate and 1. gram Calcium Carbonate	2.2
0.4 gram Magnesium Carbonate and 2. gram Calcium Carbonate	1.8
0.4 gram Magnesium Carbonate and 3. gram Calcium Carbonate	3.1
0.8 gram Magnesium Carbonate and 1. gram Calcium Carbonate	2.9
0.8 gram Magnesium Carbonate and 2. gram Calcium Carbonate	3.5
0.8 gram Magnesium Carbonate and 3. gram Calcium Carbonate	4.1

Here again it is shown that .1 gram of magnesium carbonate per 100 grams of soil entirely prevented nitrification. Neither do we observe any effective antagonism through the use of calcium carbonate.

On the one hand it was found that ammonification of dried blood was seriously interfered with by the presence of small amounts of magnesium carbonate, and on the other, nitrification was completely prevented by its presence. In neither case was there any evidence of an antagonism between magnesium and calcium carbonates. In the above nitrification experiments, magnesium carbonate not only prevented the formation of nitrates but at the same time induced a reduction in the amounts of nitrates originally present in the soil. It was observed that with the addition of magnesium carbonate a much more abundant growth of moulds took place than in the tumblers receiving calcium carbonate.

With a view of throwing further light on this question, total nitrogen was determined, both before and after the incubation period of 21 days, in a similar set of experiments to which one gram of magnesium carbonate had been added. The result showed that during the period of bacterial action, similar to that in the preceeding nitrification experiments, the soil sustained a loss equal to about 20 per cent of the combined nitrogen originally present.

Two factors suggest themselves as bearing on this question. The first and probably most important is that of volatilization and, therefore, loss of ammonia. J. G. Lipman[9] in his numerous researches found that the dilution of a heavy silt loam with silica sand caused a loss of ammonia in ammonification experiments. The loss began to manifest itself with the use of 30 per cent of sand but greatly increased with larger amounts. This loss was attributed to the volatilization of ammonia and was sufficiently great to give an appreciable odor of ammonia above the tumblers. The soils employed in the experiments herein described were largely composed of sand and contained very small amounts of silt and clay. The substances capable of fixing large amounts of ammonia are, therefore, largely absent from

[9] N. J. Sta. Rept., 1909.

these soils and consequently considerable loss may have been sustained through the volatilization of ammonia. The data on ammonification, therefore, should be considered as representing the ammonia accumulated rather than the absolute amounts formed. The relative effects of calcium and magnesium carbonates on the loss of ammonia were not investigated.

A second factor in the loss of nitrogen is that of denitrification. It was recently shown by Vogel[10] that calcium carbonate under certain conditions can bring about a considerable loss of nitrogen as nitrates in soils through denitrification. In the previous experiments it was observed that with the use of magnesium carbonate a decided reaction for nitrites could be obtained. Denitrification, therefore, took place and an actual loss of nitrogen is probably traceable to this cause. From the preceding data (Table VI) it is seen that the use of small amounts of magnesium carbonate not only inhibited nitrification but, as previously mentioned, also caused a considerable loss of the nitrates already in the soil. We have here, therefore, still further evidence of denitrification having taken place. With the use of larger amounts of magnesium carbonate, nitrification and denitrification were both inhibited but no considerable loss of the nitrates originally present in the soil took place.

It seems probable, therefore, that the smaller amounts of magnesium carbonate were toxic to the nitrifying bacteria while still allowing the denitrifiers to act, but under the influence of larger amounts of magnesium carbonate both the nitrifying and denitrifying groups were rendered inactive.

The striking nature of the results obtained in the previous ammonification and nitrification experiments suggested a study of nitrogen fixation under similar conditions. For this purpose the Anaheim soil was employed since it contains a vigorous nitrogen fixing flora. Mannite was used in these experiments and the usual method followed. The results obtained proved to be so irregular and discordant that their publication is withheld at this time. In one series a slight decrease in the amount of nitrogen fixed followed the use of magnesium carbonate, while in still another series no effects were observed.

[10] Centbl. Bakt. 34, pp. 540–561.

DISCUSSION

The experimental data presented above show that under the conditions employed and in the soils studied, calcium carbonate stimulated the ammonification of dried blood to a limited extent but exercised a more noteworthy stimulating effect on nitrification. With magnesium carbonate a pronounced toxic effect was produced. In the ammonification of dried blood there was sustained a loss of about one-third as compared with the experiments without the use of carbonates, while in the nitrification experiments magnesium carbonate completely inhibited nitrate formation. It is also noteworthy that no evidence of antagonism between calcium and magnesium carbonates was observed. It is not intended, however, to generalize from these results. It does not follow that similar results would be obtained from any soil. In fact, data already obtained from other soils show that the phenomena observed in the two soils above discussed are not of universal occurrence under similar conditions.

A further study of the lime-magnesia ratio in reference to nitrogen transformations in soils is now under way with the use of several types of Hawaiian soils and interesting results have already been obtained. A more complete interpretation of the results obtained is reserved for a subsequent publication after a wider range of observations have been made. Before a satisfactory understanding of the lime-magnesia question in regard to field crops is presented it is imperative that we have more specific knowledge concerning the effects produced on the various organisms of soils, now generally admitted to be of fundamental importance in plant growth, and it is especially important that the effects produced on the organisms affecting nitrogen transformations be more fully understood. It is hoped that this work may stimulate other investigations along this line.

The author wishes to extend his thanks to Dr. C. B. Lipman, in whose laboratory this work was carried out, for many valuable suggestions offered from time to time and the great interest shown.